DISSERTATION ON HISTOPATHOLOGICAL ANALYSIS OF ASSOCIATION OF PAPILLARY CARCINOMA THYROID WITH THYROIDITIS AND OTHER THYROID LESIONS

Dissertation submitted to

THE TAMILNADU
DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment for the award of the degree of

DOCTOR OF MEDICINE
IN
PATHOLOGY

STANLEY MEDICAL COLLEGE
CHENNAI – TAMIL NADU

MARCH 2010

CERTIFICATE

This is to certify that the dissertation entitled, **"DISSERTATION ON HISTOPATHOLOGICAL ANALYSIS OF ASSOCIATION OF PAPILLARY CARCINOMA THYROID WITH THYROIDITIS AND OTHER THYROID LESIONS"** submitted by **Dr.S.GEETHA LAKSHMI,** in partial fulfillment for the award of the degree of Doctor of Medicine in Pathology by The Tamil Nadu Dr.M.G.R.Medical University, Chennai is a bonafide record of the work done by her in the Department of Pathology, Stanley Medical College, Chennai, during the academic year 2007 – 2010.

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CONTENTS

ABBREVIATIONS

AR	-	Antigen Retrieval
DAB	-	Diamino benzidine
DPX	-	Distrene dibutyl pthalide in xylol
H & E	-	Hematoxylin and Eosin
HPE	-	Histopathological Examination
HRP	-	Horse radish peroxidase
IHC	-	Immunohistochemistry
PTC	-	Papillary thyroid carcinoma
SS Label	-	Sensitive Secondary Label
WHO	-	World Health Organization

ACKNOWLEDGEMENT

I take this opportunity to express my heart felt gratitude to **Dr.S.Chitra, M.D., Phd.,** Professor and Head of the Department of Pathology, Stanley Medical college, Chennai for her keen interest, constant encouragement , guidance and valuable suggestions throughout this study.

I take this opportunity to express my heart felt gratitude to **Dr. A.Sundaram, M.D.,** Director, Institute of Pathology, Madras Medical college, Chennai for his keen interest, constant encouragement , guidance and valuable suggestions throughout this study.

I am extremely thankful to **Dr. R. Geetha, M.D.,** Professor of Pathology, Stanley Medical college who has extended her encouragement, guidance and valuable suggestions throughout the study.

My sincere thanks to **Dr. V. Ramamoorthy, M.D.,** Professor of Pathology, Stanley Medical college for the encouragement and guidance extended to me during the study.

I am extremely thankful to **Dr. R. Padmavathy, M.D.,** Professor of Pathology, Stanley Medical college who has extended her encouragement , guidance and valuable suggestions throughout the study.

My heartfelt thanks to **Dr. P. Arunalatha, M.D.,** Professor of Pathology, Stanley Medical college for the encouragement and guidance extended to me during the study.

Last but not the least I am grateful to all the faculty members, my colleagues and the technical staff members of the Department of Stanley Medical College and **my beloved family members** for their constant support and encouragement during the period of study.

INTRODUCTION

Papillary carcinoma of the thyroid is the most common malignant tumor of the thyroid, accounting for 80% of all thyroid cancers. It occurs mostly commonly in women between 3rd to 5th decades.

The interplay between inflammatory and neoplastic disorders, firmly established in certain tissues, is a matter of controversy in the thyroid. An increased risk for developing papillary carcinoma in patients with Hashimoto's thyroiditis remains controversial ranging from 0-30%[1]

A marker found frequently in both of these thyroid disorders is described. Expression of p53 homologous nuclear protein p63 was surveyed in a spectrum of thyroid neoplasms and inflammatory disorders. p63 is postulated to regulate the stem cell phenotype in squamous epithelia, and in tumors of squamous origin[2].

This work aims at histologically classifying all the thyroid lesions and identifying the lesions of Hashimoto's thyroiditis with papillary carcinoma, and establishing the pathobiologic link between the two by immunohistochemical detection of p63 in papillary thyroid carcinoma and Hashimoto's thyroiditis.

The expression of p63 protein in Hashimoto's thyroiditis and papillary carcinoma has been studied in this work to address the postulate of a common origin from a stem cell precursor.

The study herein describes the immunohistochemical detection of p63 in papillary

carcinomas of thyroid, Hashimoto's thyroiditis and Hashimoto's thyroiditis with papillary carcinoma to find out whether p63 expression may constitute a mechanistic link between Hashimoto's thyroiditis and papillary carcinoma.

AIMS AND OBJECTIVES

- To identify various thyroid lesions and classify them according to their morphology.

- To study the occurrence of papillary carcinoma in cases of thyroiditis and other thyroid lesions.

- To establish the association between Hashimoto's thyroiditis and papillary carcinoma thyroid by immunohistochemical detection of p63 protein.

REVIEW OF LITERATURE

Thyroid cancer accounts for approximately 1% of all malignancies in developed countries with an estimated annual incidence of 122,000 cases worldwide[3].

Among epithelial tumors, carcinomas of follicular cell origin outnumber those of "C" Cell origin. Carcinomas of follicular cell origin are indolent malignancies with 10 year survivals in excess of 90% [4,5].

THE COMMONEST TUMORS AND THEIR FREQUENCIES.

Tumour type	***Frequency (%)***
Papillary carcinoma	70-85%
Follicular carcinoma	5-10%
Medullary carcinoma	5%
Malignant lymphoma	4-5%
Undifferntiated carcinoma	2-5%
Poorly differentiated (Insular carcinoma)	0.4-10%

According to surveillance Epidemiology and End results (SEER) study 10 year relative survival rates of major thyroid carcinomas are[6]

Papillary carcinoma : 0.98
Follicular carcinoma : 0.92
Medullary carcinoma : 0.80
Undifferentiated carcinoma : 0.13

GENERALIZATION ON PRIMARY THYROID CANCERS

- Papillary carcinoma is the commonest histologic type

- With the exception of angiosarcoma, tumors are 2-4 times more frequent in women than in men[7]. Female sex is usually associated with slightly better prognosis.

- Better differentiated tumors generally occur in younger patients, while the less differentiated tumors occur in older patients

- The mean ages for low, intermediate and high grade tumors are the forties, fifties and sixties respectively.

STAGING OF THYROID TUMORS (International union against cancer) Tumor, Node, Metastasis staging

Tx : Primary tumor cannot be assessed

T0 : No evidence of primary tumor

T1 : Tumor $\leq$ 2cm, limited to thyroid

T2 : Tumor >2cm but $\leq$4 cm, limited to the thyroid

T3 : Tumor >4cm, limited to thyroid or tumor any size with minimal intrathyroid or perithyriod soft tissue extension

T4a : Tumor of any size invading beyond the thyroid capsule

to invade subcutaneous tissues, larynx, trachea, esophagus or recurrent laryngeal nerve.

T4b : Tumor invades prevertebral fascia or mediastinal vessels or encases carotid artery.

Nx : Regional lymphnodes cannot be assessed

N0 : No regional lymphnode metastasis

N1 : Regional lymphnode metastasis

N1a : Metastasis to pretracheal, paratracheal, and prelaryngeal/ Delphian nodes.

N1b : Metastasis to unilateral, bilateral, or contralateral cervical or upper / superior mediastinal nodes.

DISTANT METASTASIS(M)

Mx : Distant metastasis cannot be assessed

Mo : No distant metastasis

M1 : Distant metastasis

Staging of thyroid tumors (International union against cancer)

	< 45 years old	> 45 years old
I	Any T, Any N, Mo	T1, No, Mo
II	Any T, Any N, M1	T2, No, Mo
III	–	T3, No, Mo T1/T2/T3, N1a, Mo
IVA	–	T4a, Any N, Mo T1/T2/T3, N1b, Mo
IV B	–	T4b, Any N, Mo
IV C	–	Any T, Any N, M1

Papillary carcinoma is defined as a malignant epithelial tumor showing evidence of follicular cell differentiation and characterized by distinctive nuclear features. This is the most common malignant tumor of the gland in countries having iodine sufficient or iodine excess diets[8] and comprises about 80% of thyroid malignancies. These common tumors tend to be biologically indolent and have excellent prognosis (more than 90% at 20 years)[9,10]. The tumors invade lymphatics leading to multifocal lesions and to regional lymph node metastasis. Venous invasion rarely occurs and metastases outside the neck is unusual (5-7% of cases)[11,12].

RISK ASSIGNMENT SYSTEMS ARE NOW WIDELY USED TO GUIDE TREATMENT[13,14]

Parameters assessed in AMES (Age, Metastasis, Extent of primary cancer and Tumor size) risk group definition systems.

Parameter	Low Risk	High Risk
Age	Male <40 Yrs Female <50 yrs	Male >40 years Female >50 years
Metastasis	No distant Metastasis	Distant metastasis
Extent of primary cancer	Intra thyroidal papillary carcinoma	Extra thyroidal papillary carcinoma
Size	< 5 cm	> 5cm

OTHER RISK DEFINITION - SYSTEM OR PROGNOSTIC SCORES

DAEMS

Incorporating in addition tumor DNA Content as measured by flow cytometry

AGES

Incorporating in addition tumor grade

MACIS

Incorporating in addition completeness of excision.

Papillary carcinoma can occur at any age and rarely has been diagnosed as a congenital tumor[15]. Most tumors are diagnosed in patients between 3rd and 5th decades[16, 17]. Women are affected more than men in ratios of 2:1 to 4:1[18].

Etiological factors

The addition of iodine to the diet in endemic goiter areas in Europe has been associated with a decreased incidence of follicular cancer and an increase in papillary carcinoma[19,20]. External radiation probably plays a role in the development of papillary cancer[21,22]. A great increase in the incidence of papillary carcinoma in Berlarus and the Ukraine has been apparent since the Chernobyl nuclear accident[23]. Some authors believe that patients with Graves disease have a higher than expected incidence of papillary cancer [24]. Other studies disagree[25]. Hashimoto's thyroiditis, Familial adenomatous polyposis (FAP) or Cowden disease all increase the risk for papillary carcinoma[26, 27].

Cytogenetics

Chromosomal rearrangements of receptor tyrosine kinase genes (RET and TRK)

represent the most common structural genetic alterations in papillary thyroid carcinoma. Rearrangements involving the RET gene called RET/PTC are found with highly variable frequency in 0-80% of cases.

Average incidence of RET/PTC is 20-30% in sporadic adult papillary carcinomas[28, 29] and is higher in tumors from children and young adults 45-60%[30] and in populations subjected to either accidental or therapeutic irradiation (50-80%)[31,32]. RET/PTC, is typically the most common, followed by RET/PTC 3[33,34] whereas RET/PTC 2 and novel types[35,36] account for <5% of all rearrangements.

TRK REARRANGEMENTS

Chromosomal rearrangements involving in TRK gene are found in approximately 10% of papillary carcinomas.

RAS MUTATIONS

Activating point mutation of one of the three RAS proto oncogenes occur in less than 10% of papillary carcinomas[37,38].

BRAF MUTATIONS

Point mutations of the BRAF gene at nucleotide position 1796, a thymine to adenine transversion, has been identified in a high proportion of papillary carcinomas (up to 70%)[39,40]. Papillary carcinomas have been described in patients with Familial Adenomatous Polyposis Coli, Cowden syndrome, Hereditary Nonpolyposis Colon Cancer syndrome, Peutz Jeghers

syndrome and Ataxia telangiectasia[41].

Macroscopy

Papillary carcinomas are typically infiltrative, with irregular ill defined borders and hard consistency, white to tan in colour, and a granular texture due to the presence of papillae .Cut surface is gritty because of presence of psammoma bodies and calcifications. Multifocal disease is common in ~ 65%[42, 43].

Histological appearances

The nuclei of papillary carcinoma are typically large, rounded, avoid, ground glass orphan Annie eye nuclei and grooved with small distinct nucleoli. This feature is not pathognomonic, because benign lesions such as nodular hyperplasia, follicular adenoma, Graves disease and Hashimoto's thyroiditis can exhibit clear nuclei focally[44, 45].

Nuclear grooving is not pathognomonic because it can be observed in solid cell nests, follicular neoplasms, hyalinizing trabacular adenomas, poorly differentiated thyroid carcinomas, and adenocarcinomas of non-thyroid origin[46].

Nuclear pseudoinclusions, which represent intranuclear herniation pockets of cytoplasm, are typical but not pathognomonic, are found only in a minority of tumor cells[47].

The neoplastic cells are polygonal to cuboidal, but can be attenuated, dome shaped, hobnailed or columnar. Cytoplasm is lightly eosinophilic to amphophilic but can be oxyphilic

or clear[48,49]. Stroma may show calcification or ossification[50].

The papillae encountered in nodular goiter and follicular adenoma with papillary hyperplasia are usually broad and have follicles in the cores. The lining cells are columnar with regular, non crowded, basally situated, dark, round nuclei resembling beads on string which is in striking contrast to the crowded and haphazardly oriented pale nuclei of papillary carcinoma.

The papillae that occur in thyrotoxicosis, Hashimoto's thyroiditis and toxic follicular adenoma are non branching short, stubby projections that protrude into the follicular lumen and lack well defined fibrovascular cores. In Hashimoto's thyroiditis well developed nuclear features of papillary carcinoma are lacking although nuclear clearing is common. Papillae can occur in columnar carcinoma and medullary carcinoma but the nuclear features of papillary carcinoma are lacking. Hurthle cell adenoma /carcinoma not uncommonly has a minor papillary component . The papillae are usually non arborizing and the cells do not show nuclear crowding. However occasional nuclear grooves can be present. Calcified colloid should not be mistaken for psammoma bodies.

Variants of papillary carcinoma

FOLLICULAR VARIANT OF PAPILLARY CARCINOMA

Papillary carcinoma composed entirely or almost exclusively of follicles is known as follicular variant of papillary carcinoma. Most cases grow in an infiltrative pattern. But some are encapsulated (so called Lindsay tumor). Follicles vary in size and shape, but are often elongated or irregular shaped with abortive papillary formation, colloid is usually deep staining and scalloped. Psammoma bodies and sclerosis may be present. Clinical behavior is not

different from conventional papillary carcinoma.

SOLID VARIANT

Papillary carcinoma with more than 50% solid growth[51,52] with no tumor necrosis[53,54] is known as solid variant of papillary carcinoma. It is associated with a slightly higher frequency of distant metastasis and less favourable prognosis compared with conventional papillary carcinoma.

ENCAPSULATED VARIANT

Encapsulated variant constitutes 4-14% of all papillary carcinomas. It's fibrous capsule may or may not show invasion by tumor but lymph node metastasis can occur even in the absence of capsular or vascular invasion. The prognosis is excellent.

DIFFUSE SCLEROSING VARIANT

The diffuse sclerosing variant affects children and adults. It is more aggressive than conventional papillary carcinoma, as manifested by a higher incidence of lymph node metastasis and frequent distant metastasis. Thyroid shows diffuse replacement of the parenchyma by white firm tissue which is gritty on cutting. The typical histological features include: Diffuse involvement of one or both lobes, sclerosis, heavy lymphoplasmacytic infiltrate, abundant psammoma bodies, scattered small islands of papillary carcinoma with prominent squamous or squamoid differentiation and extensive lymphatic permeation[55,56].

DIFFUSE FOLLICULAR VARIANT

This rare, aggressive form of papillary carcinoma occurs in young patients, and is characterized by diffuse involvement of the entire thyroid without formation of discernible nodules, an exclusive or predominant follicular pattern and absence of fibrosis.

MACROFOLICULAR VARIANT

These are tumors with >50% area composed of large follicles[57].

TRABECULAR VARIANT

This variant shows trabecular growth pattern in over 50% of the tumor. Tumor cells are cuboidal or columnar with the cells oriented perpendicularly in the long straight trabeculae. It is associated with poor prognosis.

CRIBRIFORM – MORULAR VARIANT

Shows a prominient cribriform pattern with interspersed squamous morules that harbour nuclei filled with lightly eosinophilic homogenous, biotin containing inclusions.

Characteristically the luminal spaces are devoid of colloid. The tumor cells are cuboidal, columnar or attenuated but can be plump, spindle – shaped forming fascicles and whorls[58]. Nuclei are often chromatin rich, but nuclear features of typical papillary carcinoma can be seen focally.

PAPILLARY CARCINOMA WITH LIPOMATOUS STROMA

In rare cases, adipocytes are interspersed within the papillary carcinoma[59].

VARIANT WITH EXUBERANT NODULAR FASCIITIS - LIKE STROMA

Stroma is composed of spindle cells lying in a vascularised fibromyxoid matrix with extravasated red cells[60].

TALL CELL VARIANT

Tumor composed predominantly of cells whose heights are at least three times their widths. More aggressive than conventional papillary carcinoma.

COLUMNAR CELL VARIANT

Characterised by mixed papillary, complex glandular, cribriform and solid pattern. The papillae are lined by tall columnar cells with hyperchromatic oval or elongated nuclei.

OXYPHILIC (ONCOCYTIC, HURTHLE CELL) VARIANT

Variant composed predominantly of cells with abundant eosinophilic granular cytoplasm due to accumulation of mitochondria.

WARTHIN TUMOR LIKE VARIANT

Papillary pattern with rich lymphoplasmacytic infiltrate in the cores of the papillae. Cells that cover the papillae often have an oxyphilic appearance and can be tall[61, 62].

CLEAR CELL VARIANT

Exhibit extensive cytoplasmic clearing , usually due to accumulation of glycogen[63].

VARIANT WITH SPINDLE CELL METAPLASIA

Papillary carcinoma can exhibit a component of spindle shaped tumor cells, which can constitute a minor to major proportion of the entire tumor[64].

DEDIFFERENTIATED PAPILLARY CARCINOMA

Co-existence of papillary carcinoma with an undifferentiated or poorly differentiated thyroid carcinoma . Prognosis is bad.

MICROCARCINOMA (PAPILLARY MICROTUMOR)

Incidentally discovered papillary carcinoma with size <1cm, but not clinically evident small sized papillary carcinomas.

IMMUNOHISTOCHEMISTRY

Stain for pan – cytokeratin, thyroglobulin, and TTF-1.

Prognostic factors in papillary carcinoma

Age : Mortality very low in patients under the age of 40 years

Sex : Male sex is associated with worse prognosis

Size : 1-1.5cm excellent prognosis. >4cm poor prognosis

Stage : Extrathyroidal extension – poor prognosis.

Tumor encapsulation : Tumor encapsulation confers a favourable prognosis.

Histological Variants **:** Tall cell, diffuse sclerosing diffuse follicular, solid, trabecular, dedifferentiated variants – more aggressive.

Completeness of tumor excision **:** Incomplete tumor excision increases the risk of recurrence.

Hashimoto's thyroiditis or chronic lymphocytic thyroiditis is the most common cause of hypothyroidism in areas of the world where iodine levels are sufficient. It is characterized by gradual thyroid failure because of autoimmune destruction of thyroid gland .The name Hashimoto's thyroiditis is derived from the 1912 report by Hashimoto describing the patients with goiter and intense lymphocytic infiltration of the thyroid (struma lymphomatosa) . This disorder is most prevalent between 45 and 65 years of age and is more common in women than in men, with a female predominance of 10:1 to 20. Although it is primarily a disease of older women, it can occur in children and is a major cause of non endemic goiter.

Epidemiologic studies have demonstrated a significant genetic component to

Hashimoto's thyroiditis, although, as in most other autoimmune disorders ,the pattern of inheritance is Non- Mendelian and is likely to be influenced by subtle variations in the functions of multiple genes. Several chromosomal abnormalities have been associated with thyroid auto immunity.

Hashimoto's thyroiditis is an auto immune disease in which the immune system reacts against a variety of thyroid antigens. There is a progressive depletion of thyroid follicular epithelial cells, which are gradually replaced by mononuclear cell infiltration and fibrosis.

Multiple immunologic mechanisms may contribute to the death of thyrocytes. Sensitization of autoreactive CD4+T-helper, cells to thyroid antigens appears to be the initiating events. The effector mechanisms for thyrocyte death include the following.

1. CD8+ cytotoxic T cell-mediated cell death

2. Cytokine-Mediated cell Death

Binding of antithyroid antibodies (anti-TSH receptor antibodies, antithyroglobulin, and antithyroid peroxidase antibodies) is followed by antibody –dependent cell-mediated cytotoxicity (ADCC).

The thyroid is often diffusely enlarged, although more localized enlargement may be seen in some cases. The capsule is intact, and the gland is well demarcated from adjacent structures. The cut surface is pale, yellow-tan, firm and somewhat nodular.

Microscopic examination reveals extensive infiltration of the parenchyma by a mononuclear inflammatory infiltrate containing small lymphocytes, plasma cells, and well-developed germinal centers. The thyroid follicles are atrophic and are lined in many areas by epithelial cells distinguished by the presence of abundant eosinophilic, granular cytoplasm, termed Hurthle cells.

This is a metaplastic response of the normally low cubodial follicular epithelium to ongoing injury. In fine-needle aspiration biopsies, the presence of Hurthle cells in conjunction with a heterogeneous population of lymphocytes is characteristic of Hashimoto thyroiditis. In "Classic" Hashimoto's thyroiditis, interstitial connective tissue is increased and may be abundant.

A fibrous variant is characterized by severe thyroid follicular atrophy and dense "Keloid-like" fibrosis, with broad bands of acellular collagen encompassing residual thyroid tissue. Unlike Riedel's thyroitidis, the fibrosis does not extend beyond the capsule of the gland. The remnant thyroid parenchyma demonstrates features of chronic lymphocytic thyroiditis.

Immunohistochemically, the follicular cells of autoimmune thyroiditis show great reactivity for keratin (particularly the high-molecular-weight for types), S-100 protein HLA-DR, and N-acetyl-a-D-galactosamine than the corresponding normal cells, their immunohistochemical profile thus resembling that of the cells of papillary carcinoma. Biochemically, the oncocytic cells of Hashimoto's thyroditis have defects of cytochrome oxidase and deletion of mitochondrial DNA.

Squamous nests, thought to arise from metaplasia of follicular cells, are common and

can reach sizable proportions. Rarely, large cysts lined by squamous epithelium and bordered by row of lymphoid follicles are seen, their appearance being highly reminiscent of branchial cleft cysts. Parenthetically, similar cysts can be also be seen in the absence of thyroiditis.

In the typical case of Hashimoto's thyroiditis, connective tissue is scanty, with slight or moderate thickening of the interlobular septa. In the fibrous variant of this disease, which comprises about 12% of all cases, fibrosis is more extensive.

The fibrous variant of Hashimoto's thyroiditis can also be confused with carcinoma when the fibrosis is associated with epithelial islands showing squamous metaplasia. Clinically, this variant characterized by a very firm goiter (often with sudden enlargement) severe pressure symptoms physical signs suggestive of cancer, and markedly elevated red cell antibody titer to thyroglobulin.

Although Hashimoto's thyroiditis typically exhibits, a diffuse appearance both grossly and microscopically, cases do occur in which a distinct nodularity is evident, the eptithelial component of the nodules having a hyperplastic quality. This could be interpreted as the combination of Hashimoto's thyroiditis and nodular hyperplasia, but in all likelihood the two abnormalities are pathogenetically related. A term such as nodular Hashimoto's thyroiditis would therefore seem more appropriate to designate this relatively common occurrence. A variation on the theme is represented by the Hashimoto's thyroiditis in which one or more distinct hyperplastic ("dominant") nodules exclusively composed of Hurthle cells having either a follicular or a solid configuration are present.

Complications of Hashimoto's thyroiditis include malignant lymphoma and lukaemia ,

papillary carcinoma and Hurthle cell neoplasms. The occurrence of striking nuclear clearing and overlapping in the follicular cells of Hashimoto's thyroiditis is of interest. There is convincing evidence for an increase in the incidence of papillary carcinoma in Hashimoto's thyroiditis but the wide variation in figures quoted suggests that the diagnostic criteria vary just as widely[65].

Recently it has been shown that RET/PTC expression can also occur in benign thyroid lesion like Hashimoto's thyroiditis[66]. Several authors have suggested an association between Hashimoto's thyroiditis and papillary carcinoma[67,68].

The RET/PTC rearrangement is highly specific for papillary thyroid carcinoma and is associated with characteristic nuclear features seen in papillary thyroid carcinoma. There is an overlap in the morphological features, immunohistochemical staining pattern, and most importantly, molecular profile between papillary thyroid carcinoma and Hashimoto's thyroiditis. Hashimoto's thyroiditis almost always harbours a genetic rearrangement that is strongly associated with and is highly specific for papillary thyroid carcinoma. Submicroscopic foci must be present in Hashimoto's thyroiditis , although the clinical behavior is still benign[69].

It has been established that papillary thyroid carcinoma is more frequent in Hashimoto's thyroiditis and also that the increased incidence of occult microscopic papillary thyroid papillary carcinoma is high[70].

PTC associated proteins like GAL3, CITED, CK19, FN1 and HBME1 are focally expressed in Hashimoto's thyroiditis in thyrocytes with PTC like nuclear changes suggesting shared molecular features between these thyrocytes and PTC.

These changes are quantitatively different, being diffuse in PTC and focal in Hashimoto's thyroiditis, raising the possibility of activation of PTC associated genes in latter leading to premalignant transformation in cases of Hashimoto's thyroiditis[71,72].

It is hypothesized that papillary thyroid carcinomas, which may be associated with squamous metaplastic changes, might express p63, whereas other thyroid neoplasms that lack the capacity for squamous differentiation might be p63 negative. A high percentage of p63 positive foci has been described in most cases of papillary carcinoma thyroid and Hashimoto's thyroiditis, in contrast with the uncommon to absent p63 expression in other primary thyroid neoplasms. These findings raise the possibility that p63 expression may constitute a mechanistic link between Hashimoto's thyroiditis and papillary carcinoma.[73]

p63

p63 gene is structurally similar to the p53 gene, consisting of 5' region that codes for a protein moiety that activates transcription of same genes activated by p53, a central sequence coding for a DNA-binding region, and a3' sequence coding for an oligomerization-promoting region.

The p63 gene codes for 6 protein isoforms based on alternate splicing and the existence of 2 promoters, one conventional, the other a biologically active internal promoter that generates truncated p63 proteins that fail to activate transcription and act as dominant negative blockers of p53 protein actions.[74]

p63 has been postulated to have a critical role in maintaining the integrity of squamous and other epithelia by maintaining the balance between basaloid stem cell commitment to undergo amitotic differentiation versus retention of a dividing undifferentiated stem cell phenotype.

To date p63 expression has been demonstrated in basal layers of squamous epithelia, urothelium, basal layers of prostate gland epithelia, myoepithelial cells of, submucosal gland epithelia and in the basal reserve cells of ciliated bronchial epithelia,[75].

p63 is hypothesized to play an important role in maintaining the epidermal stem cell population. Immunohistochemical analyses show p63 protein localization and expression in basal /progenitor cells of several epithelial tissues such as epidermis mammary glands, prostate and urogenital tract. p63 expression is lost as these cells migrate from the basal layer and

become terminally differentiated cells.

In thyroid, it is hypothesized that papillary thyroid carcinomas and Hashimoto's thyroiditis which may be associated with squamous metaplastic changes, might express p63 whereas other thyroid neoplasms that lack the capacity for squamous differentiation might be p63 negative[76].

IMMUNOHISTOCHMISTRY

Immunohistochemistry (IHC) involves two disciplines – immunology and histology.

Immunohistochemistry is used to determine expression of particular antigen and its micro anatomic location in the tissue. IHC uses antibodies to distinguish the antigenic differences between the cells. The differences can specifically identify the lineage of cell population and define biologically distinct populations of cells within the same lineage.

Immunohistochemistry started in 1940 when Coons developed all immunofluoresence technique to detect corresponding antigens in frozen sections.

Taylor and colleagues in 1947 showed that it was possible to demonstrate antigens in routinely processed tissue. Antigen retrieval technique was introduced by Shi and associates in 1991. It is a simple method that involves heating paraffin processed sections at high temperatures before IHC staining.

The use of antibody in IHC depends upon the sensitivity and specificity of the antigen – antibody reaction and the Hybridoma technique provides limitless source of highly specific antibodies.

BLOCKING NON-SPECIFIC BACKGROUND STAINING

Background staining is due to either non-specific binding or presence of endogenous enzymes. Non specific binding with polyclonal primary antibody is minimized by pre-

incubating sections with serum from same species on optional working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues is abolished by peroxidase blocking or by using alternate systems such as immunogold technique.

Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for 10 min at room temperature (almost complete abolition of endogenous peroxidase activity). Endogenous alkaline phosphatase is blocked by addition of 0.1M concentration of levamisole to the enzyme substrate solution.

DETECTION SYSTEMS

Antibodies are labeled or flagged by some method to permit visualization these include fluorescent substances, enzymes forming coloured reaction with suitable substrate (light microscopy) or heavy metals (Electron microscopy).

METHODS OF IHC

DIRECT LABELING METHOD

Antibody is attached with a label by chemical means and directly applied to tissue sections. It is a rapid and easy procedure and caries the disadvantage of detection of multiple antigens which require separate incubation with respective antibodies.

INDIRECT LABELING METHOD

Enzymes are labeled with the secondary antibody, which is produced against primary antibody. This method is more sensitive and easy to handle. The advantages also include increased versatility, higher working dilution of primary antibody, secondary antibodies against primary antibodies of a different species easy to prepare.

AVIDIN – BIOTIN TECHNIQUES

High affinity binding between biotin and avidin is used in this procedure. Biotin is chemically linked to primary antibody and avidin is conjugated chemically to enzyme. The avidin binds to biotinylated antibody thus localizing the peroxidase moiety at the site of antigen.

Disadvantage of this technique is that endogenous biotin produces nonspecific background staining.

AVIDIN BIOTIN CONJUGATE PROCEDURE

In this technique primary antibody is added followed by biotinylated secondary antibody and next by preformed complexes of avidin and biotin horse radish peroxidase conjugate. This is a more sensitive method.

BIOTIN STREPTAVIDIN SYSTEM

Streptavidin is used in place of avidin. Streptavidin complexes are more stable.

IMMUNOGOLD SILVER STAINING TECHNIQUE

This is used in ultrastructural immunolocalisation. Gold particles are enhanced by the addition of several layers of metallic silver. The fine silver deposits in the background create confusion when small amounts of antigen are identified.

POLYMERIC METHOD

This technique permits binding of large number of enzyme molecules to secondary antibody via the Dextran backbone. The advantages of this technique are increased sensitivity, minimized non specific background staining and a reduction in the total number of assay steps.

FIXATION

This is a critical step as the preservation of morphology is essential for interpretation of IHC. 10% buffered neutral formalin is commonly used because of the following advantages:

1. Good morphological preservation.
2. Cheap.
3. Sterilizes tissues.
4. Carbohydrate antigens are better preserved.

The disadvantage of masking of antigens during fixation can be overcome by antigen retrieval technique.

ANTIGEN RETRIEVAL

This procedure involves unmasking of the antigens. The following techniques can be used.

1. Proteolytic enzyme digestion.
2. Microwave antigen retrieval
3. Microwave and trypsin antigen retrieval technique.
4. Pressure cooker antigen retrieval

MATERIAL AND METHODS

SOURCE OF DATA

Thyroidectomy (Hemi, Subtotal and total thyroidectomy) cases received in the Department of Pathology, Stanley Medical College from the Department of Surgery during the study period from May 2008 to September 2009

INCLUSION CRITERIA

All cases of Hashimoto's thyroiditis, papillary carcinoma and Hashimoto's thyoiditis with papillary carcinoma irrespective of patient's age and sex were included in the study.

EXCLUSION CRITERIA

All other thyroid lesions and cases with poor clinical data were excluded from the study.

METHOD OF DATA COLLECTION

A total of 195 cases received in the department during the study period were taken for study. The tissues so obtained were processed and sections were cut at 5 microns using Shandon semiautomatic microtome. Hematoxylin and eosin staining of the sections were done and various histomorphological changes were studied. Necessary photomicrographs were taken.

Histopathological diagnosis of the specimens studied included adenoma, adenomatous goitre, nodular goitre, colloid goitre, toxic goitre, multinodular goitre, Hashimoto's thyroiditis and papillary carcinoma.

Out of 195 cases, 72 cases were taken for study out of which 38 cases were Hashimoto's thyroiditis, 7 cases were Hashimoto's thyroiditis with papillary carcinoma and 27 cases were papillary carcinoma . 21 cases were selected randomly, 7 cases from each of the above three category for immunohistochemical study of p63 immunoprotein status. Section from cervix was taken as control to study p63 immunoprotein status(Fig.25,26). Only 21 cases were studied because of the cost effectiveness in doing immunohistochemical studies.

METHOD OF TISSUE PREPARION FOR IHC

10% neutral buffered formalin was used for fixing the specimens, the tissues were processed in various grades of alcohol and xylene using automated histokinette. Paraffin blocks were prepared and sections of 5 microns thickness were cut using Shandon semi automatic microtome using disposable blades and stained with hematoxylin and eosin. Suitable blocks were chosen for IHC.

Sections for immunohistochemistry were also cut in semiautomatic microtome using disposable blades. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using the microwave technique using EDTA (PH 8) buffer solution and then treated by HPR (Horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following stages.

- Treatment with peroxidase block-for inhibiting endogenous peroxidases in the tissue for 20 minutes.

- Washed in TRIS buffer for 5 minutes.

- Application of power block- to block non specific antigen antibody reaction – 20 minutes.

- Blot dried the excess power block.

- Application of primary antibody for 60 minutes.

- Washed in TRIS buffer for 5 minutes thrice.

- Application of super enhancer for 30 minutes which enhanced the final reaction product by increasing the sensitivity of antigen antibody reaction.

- Application of SS label – secondary antibody from goat with the tagged horse radish peroxidase enzyme for 30 minutes.

- Wash thrice in TRIS buffer.

- Application of DAB (Diamino benzidino) chromogen for 5 minutes – this was cleaved by the enzyme to give the coloured product at the antigen sites.

- Washed in distilled water for 5 min.

- The slides were counterstained with hematoxylin.

Air dried and mounted with DPX (Distrene dibutyl pthalide in xylol).

p63 nuclear staining pattern and staining intensity ranged from strong to moderate to weak. Staining was focal rather than extensive or confluent. Extent of staining varied from multiple positive foci to scattered or rare foci.

METHODS OF SCORING

Included measurements of the intensity of staining or percentage of positive cells or a combination of the two.

SCORE FOR INTENSITY OF STAINING OF p63

- 0-no staining
- 1-weak staining
- 2-moderate staining
- 3-strong staining

Given a maximum score of 300 if 100 percent of tumor cells show strong positivity

SCORE FOR PROPORTION OF POSITIVE CELLS

- 0- less than 5% positive nuclei
- 1- 5 to 25% positive nuclei
- 2- 26 to 75% positive nuclei
- 3- over 75% positive nuclei

PERCENTAGE METHOD

Positivity of cells was defined regardless of staining intensity. More than 10% of positive cells represented the cut off between negativity and positivity[76].

OBSERVATION AND RESULTS

A total of 195 biopsy samples were studied. The various thyroid lesions were classified based on the morphology

NON NEOPLASTIC LESIONS

- Adenomatous goiter : 33
- Multinodular goiter : 37
- Colloid goiter : 26
- Hashimoto's thyroiditis (Fig.1-4) : 38
- Toxic goiter : 3

NEOPLASTIC LESIONS

- Follicular adenoma : 23
- Hashimoto's thyroiditis

  with Papillary carcinoma (Fig.5-12) : 27

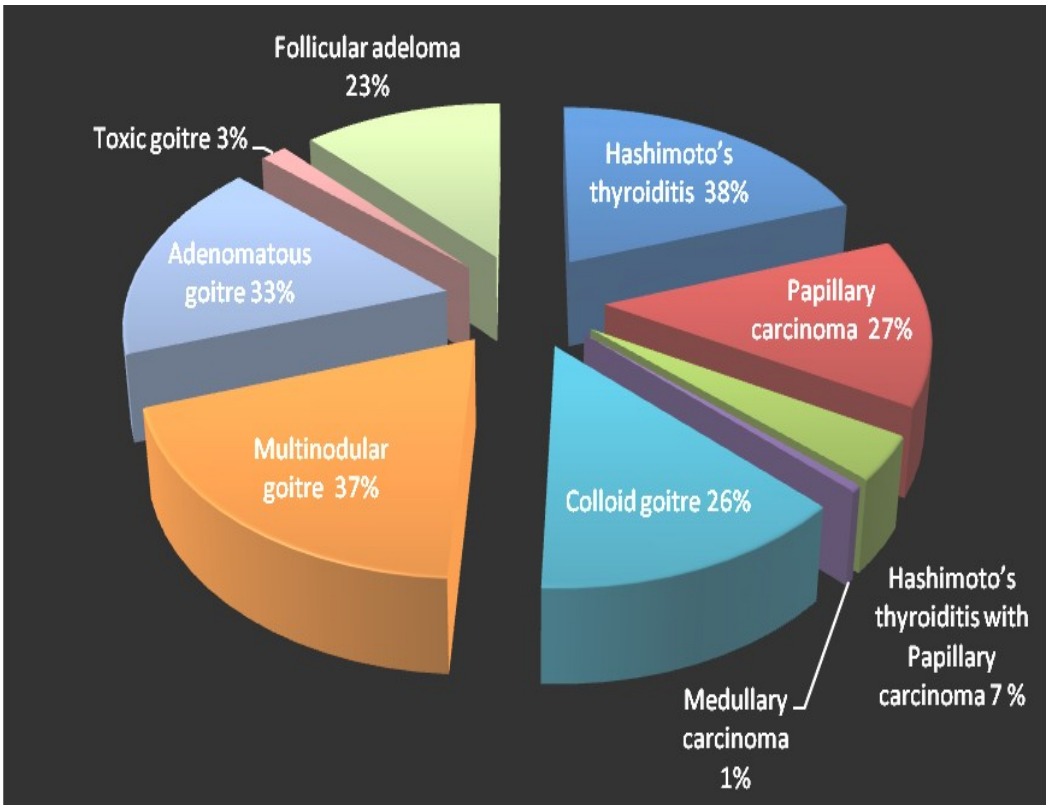
- Papillary carcinoma (Fig.13-24) : 7
- Medullary carcinoma : 1

CASE DISTRIBUTION IN DIFFERENT CATEGORIES

TABLE NO - 1

Category	No of cases
Hashimoto's thyroiditis	38
Papillary carcinoma	27
Hashimoto's thyroiditis with Papillary carcinoma	7
Medullary carcinoma	1
Colloid goiter	26
Multinodular goiter	37
Adenomatous goiter	33
Toxic goiter	3
Follicular adenoma	23
TOTAL	195

CASE DISTRIBUTION IN DIFFERENT CATEGORIES



AGE DISTRIBUTION OF THYROID LESIONS

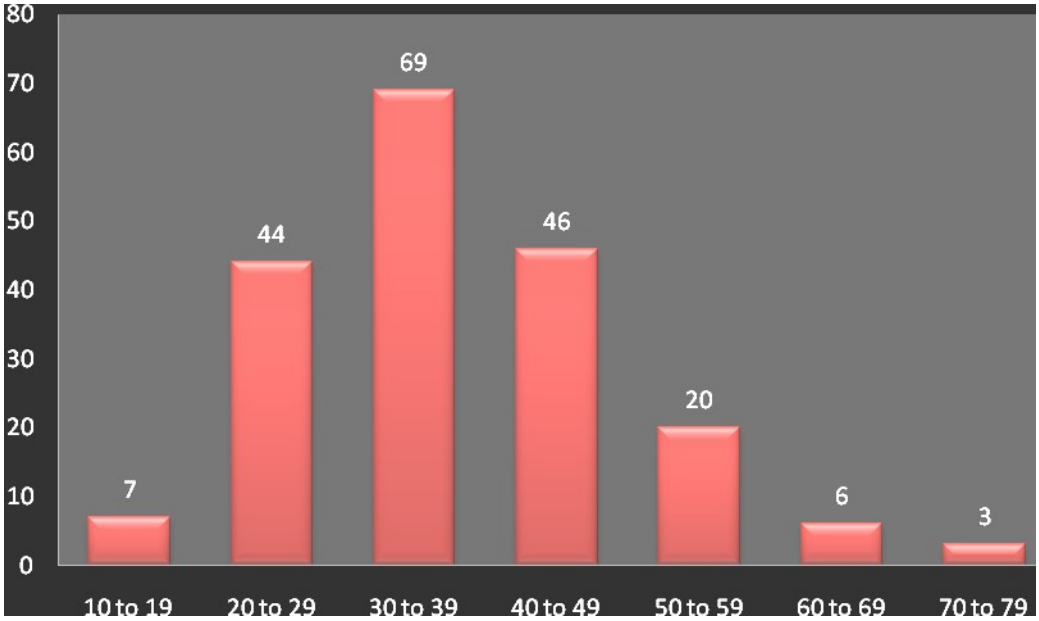
TABLE NO - 02

Age	No of cases
10 to 19	7
20 to 29	44
30 to 39	69
40 to 49	46
50 to 59	20
60 to 69	6
70 to 79	3
TOTAL	195

AGE DISTRIBUTION OF THYROID LESIONS

Age range was from 16 to 75 years. Majority of cases belonged to 30 to 39 years age group followed by 40-49 years age group

AGE DISTRIBUTION OF THYROID LESIONS



SEX DISTRIBUTION

Out of 195 cases, 178 were female patients and 17 were male patients.

IMMUNO HISTOCHEMISTRY RESULTS:

Immunohistochemical analysis of p63 was done in 21 cases which included 7 cases of Hashimoto's thyroiditis, 7 cases of papillary carcinoma and 7 cases of Hashimoto's thyroiditis with papillary carcinoma. Cases were selected randomly.

p63 immunostain staining intensity was observed and tabulated as follows:-

TABLE NO - 3

S.No	Biopsy No.	HPE Diagnosis	p63 reactivity
1.	20/08	Hashimoto's thyroiditis	Weak
2.	69/08	papillary carcinoma	Strong
3.	782/08	Hashimoto's thyroiditis	Negative
4.	816/08	Hashimoto's thyroiditis	Negative
5.	935/08	Hashimoto's thyroiditis	Negative
6.	2156/08	Hashimoto's thyroiditis with papillary carcinoma	Negative
7.	2273/08	Hashimoto's thyroiditis with papillary carcinoma	Negative
8.	2702/08	Hashimoto's thyroiditis	Negative
9.	2729/08	Hashimoto's thyroiditis with papillary carcinoma	Negative
10.	3197/08	Hashimoto's thyroiditis with papillary carcinoma	Negative
11.	3340/08	papillary carcinoma	Moderate
12.	3602/08	papillary carcinoma	Weak
13.	3872/08	Hashimoto's thyroiditis with papillary carcinoma	Negative
14.	153/09	Hashimoto's thyroiditis	Negative
15.	797/09	Hashimoto's thyroiditis	Negative

S.No	Biopsy No.	HPE Diagnosis	p63 reactivity
16.	831/09	papillary carcinoma	Strong
17.	868/09	papillary carcinoma	Moderate
18.	2494/09	papillary carcinoma	Moderate
19.	2535/09	Hashimoto's thyroiditis with papillary carcinoma	Negative
20.	2730/09	Hashimoto's thyroiditis with papillary carcinoma	Negative
21.	2923/09	papillary carcinoma	Weak

The above results show that all isolated cases of papillary carcinoma showed weak to strong reactivity of p63 (Fig.31-34). One case of Hashimoto's thyroiditis showed occasional weak reactivity (Fig.27-30) and all cases of Hashimoto's thyroiditis with concurrent papillary carcinoma and other cases of Hashimoto's thyroiditis were negative.

The theory of common precursor stem cell origin of Hashimoto's thyroiditis and papillary carcinoma could not be confirmed. Similar previous studies (73) had shown equivocal results and stated that p63 positivity, although common to papillary carcinoma, Hashimoto's thyroiditis and thyroid cells with squamoid features, the possible mechanistic role in linking these various entities could not be proved.

DISCUSSION

Papillary carcinoma of the thyroid is the most common malignant tumor of the thyroid accounting for 80% of all thyroid cancers. Overall, it accounts for 1 % of all cancers.

These tumors may arise at any age but are most common between 3-5th decades[16,17] and twice as common in women[18].

The linkage of Hashimoto's thyroiditis to papillary thyroid carcinoma is a matter of controversy. The increased risk of developing papillary carcinoma in patients with Hashimoto's thyroiditis ranges from 0-30% [1]

In the present study, a total of 195 samples were studied and various lesions were classified based on histomorphology.

Based on histomorphology non neoplastic lesions outnumbered neoplastic lesions and all the thyroid lesions were found to be more common in females.

Out of 195 cases 72 cases were taken for study. Of 72 cases, 27cases were papillary carcinoma and 45 cases were Hashimoto's thyroiditis out of which 7 cases of Hashimoto's thyroiditis were associated with papillary carcinoma.

Among the non neoplastic lesions Hashimoto's thyroiditis was incidentally found to be more commonly associated with papillary carcinoma than others.

An attempt was made here to ascertain the possible association between Hashimoto's thyroiditis and papillary carcinoma.

The possible association between Hashimoto's thyroiditis and papillary carcinoma was studied in cases of Hashimoto's thyroiditis, Hashimoto's thyroiditis with papillary carcinoma and papillary carcinoma. 21 cases were selected randomly which included equal number of cases from each of the above three categories. p63 immunoprotein status was studied in these cases.

In the current study, p63 was detected in all cases of papillary carcinoma. Staining intensity ranged from strong to moderate to weak to moderate but distinct. Staining was focal rather than extensive or confluent. Extent of staining varied from multiple positive foci to scattered to rare foci.

One case of Hashimoto's thyroiditis showed occasional focal p63 staining in follicle like groupings.

All cases of Hashimoto's thyroiditis with concurrent papillary carcinoma were p63 negative.

The results obtained from this study also co-rrelates with the previous literature results (Table - 4)

Name of the study	No of cases of hashimoto's thyroiditis	No of cases of papillary carcinoma	No of cases of hashimoto's Thyroiditis with papillary carcinoma	Hashimoto's thyroiditis	Papillary carcinoma	Hashimoto's with Papillary carcinoma
Pamela unger et. al . Human pathology 2003	13	33	17	9/13 (69.2%)	27/33 (81.8%)	15/17 (88.2%)
Jorge S et.al . Modern pathology 2003	–	6	–	–	6/6 (100)	–
Dina BL Demellawy et.al diagnostic pathology	–	75	–	–	52/75 (70%)	–
Present study	38	27	7	1/38 (0.026%)	27/27 (100%)	–

These studies have also shown equivocal results regarding the p63 immunoprotein expression in cases of Hashimoto's thyroiditis with papillary carcinoma. Therefore the association between Hashimoto's thyroiditis and papillary carcinoma remains ambiguous.

Thus p63 positivity, although common to papillary carcinoma, Hashimoto's thyroiditis and thyroid cells with squamoid features, a mechanistic role in linking these various entities cannot be proved because of the following reasons.[73]

- Failure to explain the existence of p63 positive papillary carcinomas that occur in the absence of Hashimoto's thyroiditis.

- Failure to account for p63 negative cases of Hashimoto's thyroiditis.

- Failure of expression of p63 in all cases of Hashimoto's thyroiditis with concurrent papillary carcinoma.

Therefore the theory of common precursor stem cell origin of Hashimoto's thyroiditis and papillary carcinoma could not be confirmed in this study and molecular analysis are needed for definite confirmation.

SUMMARY AND CONCLUSION

A total of 195 thyroidectomy specimens were received during the period of study from May 2008 to September 2009, and all lesions were studied histomorphologically. Immounhistochemical analysis of p63 status was done to study the association between Hashimoto's thyroiditis and papillary carcinoma.

The thyroid lesions were divided into to neoplastic and non neoplastic lesions and were further classified histologically.

Immonohistochemical analysis of p63 status was done in 21 cases selected randomly from cases of Hashimoto's thyroiditis, Hashimoto's thyroiditis with papillary carcinoma and papillary carcinoma.

p63 positivity was found in all cases of papillary carcinoma, occasionally in Hashimoto's thyroiditis and absent in Hashimoto's thyroiditis with concurrent papillary carcinoma.

The possible association between Hashimoto's thyroiditis and papillary carcinoma could not be established because of occurrence of p63 negative cases of Hashimoto's thyroiditis and lack of expression of p63 in all cases of Hashimoto's thyroiditis with concurrent papillary carcinoma. Therefore molecular analysis is required for further confirmation of their association.

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